

IN THE SPECIFICATION:

Kindly amend the specification as follows, in accordance with 37 C.F.R. § 1.121:

[0005] In recent years, proteins have been identified that have a function of specifically secreting an L-amino acid to the outside a cell of microorganism, as well as genes which encode these proteins. In particular, Vrljic et al. have identified a gene involved in secretion of L-lysine derived from *Corynebacterium glutamicum* R127 to the outside of a cell (Vrljic M., Sahm H., Eggeling L., Molecular Microbiology 22:815-826 (1996)). This gene was designated as lysE, and it was reported that L-lysine producing ability of *Corynebacterium* bacteria could be improved by enhancing the expression of this gene in *Corynebacterium* bacteria (WO97/23597). The gene lysE is known to secrete not only L-lysine, but also L-arginine (Bellmann A., Vrljic M., Patek M., Sahm H., Kramer R., Eggeling L. Microbiology, 147:1765-1774 (2001)). It is also known that production of some L-amino acids can be improved by increasing expression amounts of amino acid secreting proteins in *Escherichia coli* (Japanese Patent Laid-open No. 2000-189180). For example, it is reported that production of cysteinecystine, cysteine, and so forth can be improved by enhancing the expression of ORF306 gene in *Escherichia coli* (EP885962).

[0008] It is a further object of the present invention to provide a bacterium belonging to the genus *Methylobacillus*, into which a DNA which is able to be expressed is introduced, and said bacterium having an ability to produce L-lysine or L-arginine, wherein said DNA encodes a variant of a protein, the protein having a loop region and six hydrophobic helixeshelices and is involved in secretion of L-lysine to the outside of a cell, and wherein said variant does not contain said loop region and facilitates secretion of L-lysine, L-arginine or both to the outside of a methanol-assimilating bacterium when said DNA is introduced into said methanol-assimilating bacterium.

[0009] It is even a further object of the present invention to provide the bacterium as described above, wherein said mutant protein substantially consists of only the hydrophobic helixeshelices.

[0010] It is even a further object of the present invention to provide the bacterium as described above, wherein said variant has six hydrophobic helixeshelices.

[0011] It is even a further object of the present invention to provide the bacterium as described above, wherein said variant is a complex comprising a peptide containing the first, second, and third hydrophobic helixes-helices relative to the N-terminus, and a peptide containing the fourth, fifth, and sixth hydrophobic helixes-helices relative to the N-terminus.

[0029] The *Methylobacillus* *bacterium* of the present invention can be obtained by introducing a DNA encoding a variant of a protein having a loop region and six hydrophobic helixes which is involved in secretion of L-lysine to the outside of a cell, whereby the DNA has a mutation which results in deletion of the loop region, and/or results in the protein variant substantially consisting of only the hydrophobic helixeshelices. The expression “substantially consisting of only the hydrophobic helixes” means that the mutant LysE is completely deficient in the loop region or deficient in most of the loop region to such an extent that the function of the mutant LysE should not be affected.

[0032] The inventors of the present invention found that the *lys* gene was lethal in a methanol-assimilating bacterium, but that a DNA encoding a variant of the LysE protein that did not have the loop region or substantially consisted of only the hydrophobic helixeshelices, increased the secretion of L-lysine and/or L-arginine to the outside of a cell of methanol-assimilating bacterium. The DNA of the present invention encodes such a mutant LysE protein that does not have the aforementioned loop region, or that substantially consists of only the hydrophobic helixeshelices.

[0033] The aforementioned mutant LysE is not particularly limited so long as it has one or more hydrophobic helices and when expressed results in increased secretion of L-lysine, L-arginine or both when it is introduced into a methanol-assimilating bacterium. Specifically, a DNA encoding a mutant LysE that has all of the first to sixth hydrophobic helices from the N-terminus is encompassed. More specifically, a DNA encoding a peptide containing the first to third hydrophobic helices relative to the N-terminus, and encoding a peptide containing the fourth to sixth hydrophobic helices relative to the N-terminus is encompassed. The aforementioned *lysE24* is an example of the mutant *lysE* that encodes a peptide containing the first to third hydrophobic helices and a peptide containing the fourth to sixth hydrophobic helices. The *lysE24* gene is introduced by a mutation with a stop codon downstream from the region encoding the third hydrophobic helix. When a region downstream from this stop codon was deleted as described in the examples, the mutant *lysE24* gene did not cause L-lysine to accumulate in the medium when expressed in *Methylobacillus glycogenes* NCIMB 11375 strain. Therefore, it is estimated that a peptide containing the first to third hydrophobic helices and a peptide containing the fourth to sixth hydrophobic helices are separately translated and function in *Methylobacillus glycogenes*. The results show that introduction of the *lysE24* gene into a *Methylobacillus* bacterium will result in improvement of the production of L-lysine or L-arginine.

[0038] The reference WO97/23597 discloses *lysE*, and only shows the *lysE* gene of coryneform bacterium introduced into a coryneform bacterium. Furthermore, it only mentions L-lysine as the secreted amino acid, and discloses a novel protein secretion system, including LysE having a structure containing six transmembrane helices. However, the inventors of the present invention confirmed that LysE derived from

coryneform bacteria did not function at all in methanol-assimilating bacteria.

[0039] Furthermore, the obtained factor is a novel L-lysine secretion factor, which has a basic structure different from the known LysE of coryneform bacteria having six transmembrane helices on one polypeptide, and this factor can no way be anticipated from the disclosure of the aforementioned patent specification that discloses LysE.

[0091] This mutant *lysE* gene was designated as *lysE24* gene. When the nucleotide sequence of *lysE24* gene was analyzed, it was found that this mutation did not result in an amino acid substitution, but a nonsense mutation introducing a stop codon around the center of the translation region of *lysE*. It has been reported that the *lysE* gene of *Corynebacterium* bacteria encodes a membrane protein having six hydrophobic helices (helices (Vrljic M., Sahm H., and Eggeling L., Molecular Microbiology 22:815-826 (1996)). In contrast, it was found that since the above *lysE24* gene contained a stop codon, the protein encoded by this gene had a structure different from that of the wild-type LysE protein. As a result, the LysE mutant functioned in *Methylophilus* bacteria due to this structure.